Current Research on the Safe Processing of Acidified Foods

Fred Breidt
USDA/ARS Raleigh, NC

NC Food Safety and Defense Task Force
May 2-3, 2012

USDA/ARS
Food Science Research Unit

- Located within the Food, Bioprocessing and Nutrition Sciences Dept., NC State Univ.
  - Four faculty: vegetable acidification, fermentation, as well as sweet potato processing
- Focus on processing technology and safety
- Since 1935. All publications are now available online.
- Since 2005, ARS Natl. Program project on the safety of acidified foods.

Research priorities

- Prevent an outbreak of pathogenic bacteria in acid and acidified vegetable products…
  - What is the greatest threat?
  - What is the likelihood of occurrence?
- Science based regulation
  - Fill in knowledge gaps
    - Industry needs and regulatory questions
  - Novel ways of producing safe products?
- Fundamental knowledge about pathogens in acidified foods.
  - Acid resistance and survival of pathogens in acid and acidified foods

Current projects

- Applied research (*E. coli, Salmonella,* and *Listeria*)
  - Thermal processing at pH 4.6
  - Cold-Fill-Hold studies at pH 3.5
  - Alternative acids (citric, phosphoric, preservative acids)
  - Spore-forming bacilli, pH increase?
- Basic research (primarily *E. coli* O157:H7 and related serotypes)
  - Modeling internal pH, charge/ion balance
  - Internal cell metabolites
  - Acid resistance of alternate *E. coli* serotypes (O104:H4)
  - Modeling buffer capacity

Additional funding and support

- National Integrated Food Safety Initiative: *Bridging the Gap: Integrated Research and Extension in Support of Small Processors of Acidified Canned Foods*, 3Yr
  - Some funds for research to fill the knowledge gaps: cold fill hold, thermal processing, and bacillus spoilage (pH rise)
  - Project Investigators (PI's): Dr. Barbara H. Ingham (Lead) & Dr. Fletcher Arritt
- Collaborator with Dr. David Green: *Assisting the Integrated Food Safety System's National Food Training Program*, 3 Yr.
  - FDA Training (curriculum committee)
- Direct industry support

Three big questions

1. What conditions are needed for thermal processing acidified foods at 4.6?
   - Vegetative pathogen kill at pH 4.6
   - Thermal processing for spores with organic acids
2. Can bacillus spores germinate and raise pH under anaerobic conditions in a variety of acidified vegetables?
   - What is the mechanism of pH increase?
   - Role of oxygen
   - Buffering
3. Can ‘reasonable’ cold fill hold conditions at pH 3.5 and 10^{o}C (50^{o}F) be identified?
   - Different organic acids and concentrations

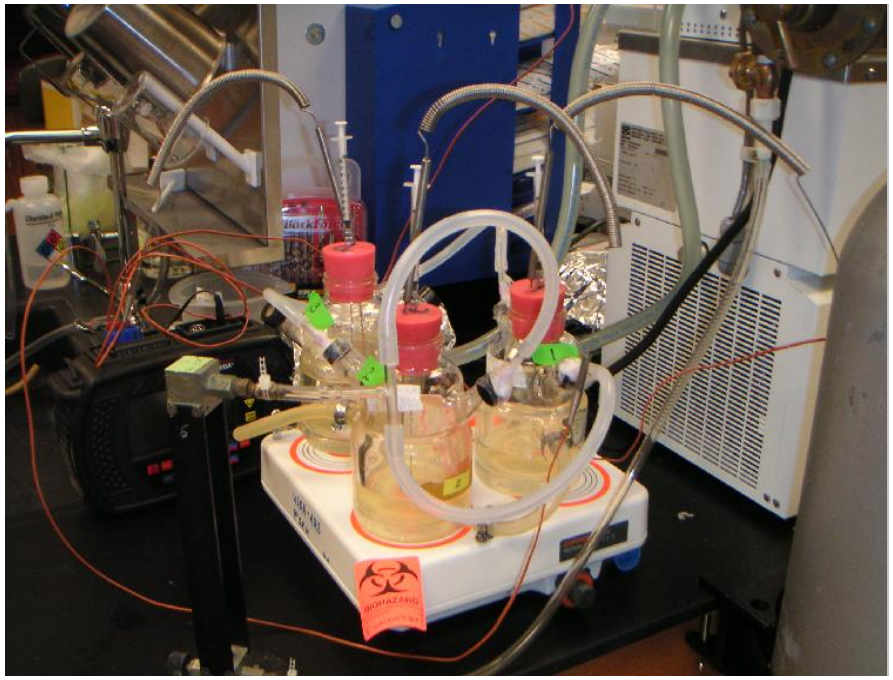
1. Thermal processing at pH 4.6

- Current published data is for pH 4.1
  - Acidified pickles
  - Cucumbers juice as a "generic" vegetable medium
- Vegetative cells *vs.* Spores (questions 1 & 2)
  - Tomato products, bad seals, and FDA concerns?
  - Industry knowledge? (Fred.Breidt@ars.usda.gov)
  - Effects of organic acids on 5D kill of vegetative pathogens
  - pH effect vs. organic acids
- Another important question: *Is a 5-D kill the right target to shoot for?*
  - Risk assessment approach?
  - Not just for thermal processing!

Thermal processing: microbiological methods

- Use a cocktail of acid resistant EHEC strains
  - Most heat/acid resistant in vegetable broth medium
- Induce acid resistance
  - Static growth at 37^{o}C
- Cucumber juice medium
  - Non-inhibitory
  - pH 4.6, 0.6% acetic acid
- Use non-selective media for plating cells
- Independent replication

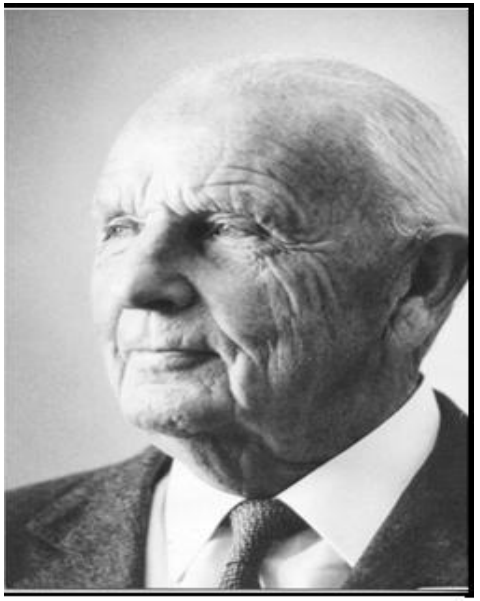
The *E. coli* inquisition…



Modeling approach

1. Generate Log CFU/ml *vs.* time data
2. Determine 5D reduction value and the standard error (SE) using a version of the Weibull model

   Note: Dr. Jason Osborne, NCSU statistics
3. Plot the $\log_{10}$ (5D + 5xSE) *vs.* Temperature to determine Z value
4. Determine the survival a reference temperature of 160^{o}F (F_{160})



Wallodi Weibull 1887-1979
Photo by Sam C. Saunders

pH 4.1 or lower:

TABLE 2. Z and F Values

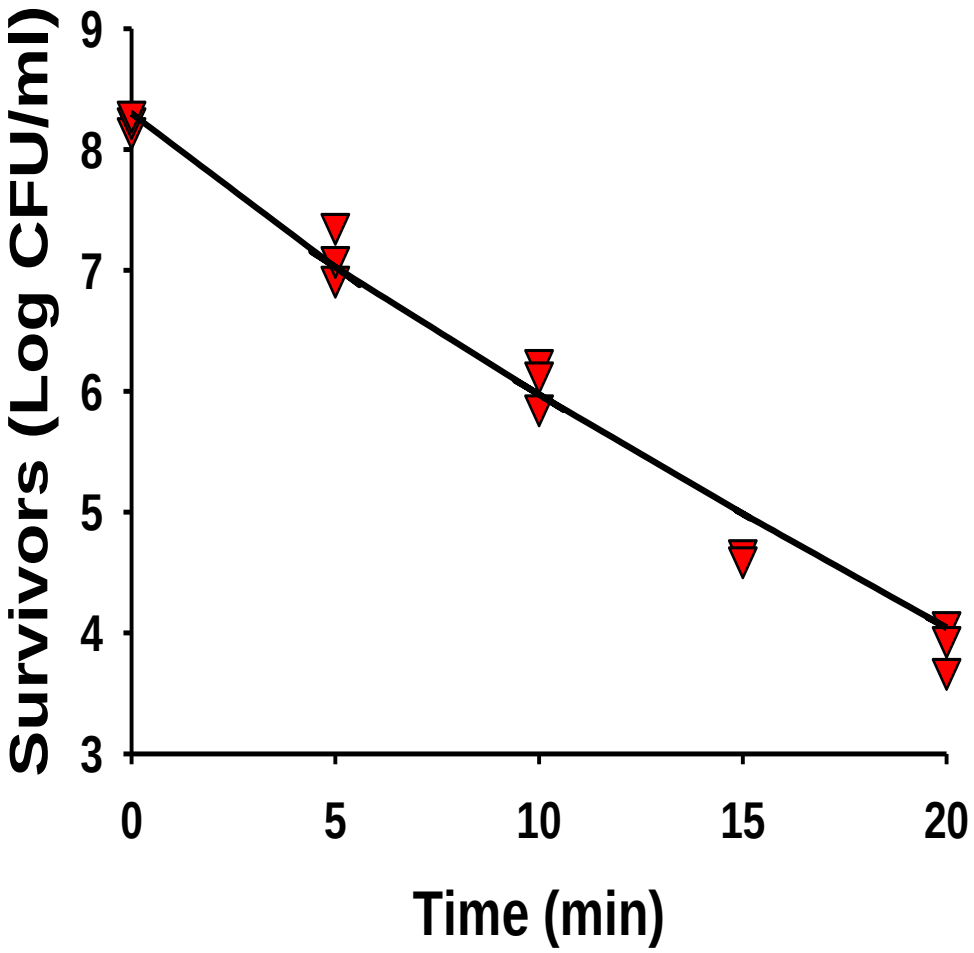
Model[a]	Z value (°F)	F_{160}[b]
Exp. Decay (5SE)	19.50	1.20
Exp. Decay	15.70	0.34
Five D Model	11.98	0.08
One D Model	11.98	0.02

[a]Models as described in the text: Exp. Decay (5SE), exponential decay model with five times the standard error added; Exp. Decay, exponential decay model; Five D Model, linear model based on a five log reduction; One D Model, linear model based on a one log reduction.

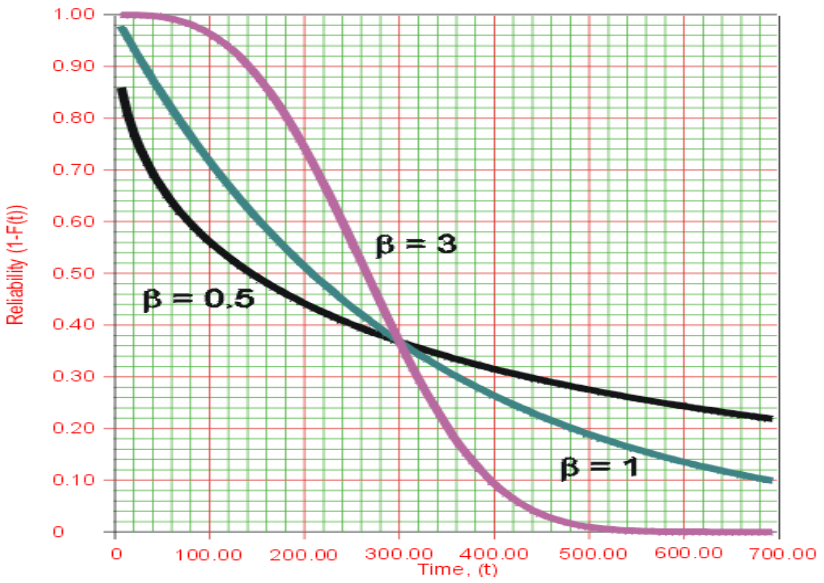
[b]F_{160}: Time in minutes (F value) needed to achieve the predicted reduction in cell numbers at a reference temperature of 160°F.

Breidt F, Sandeep KP, Arritt F. 2010. Use of Linear Models for Thermal Processing of Acidified Foods. *Food Prot Trends* 30(5):268-272.

TDT data 64ºC (147ºF)



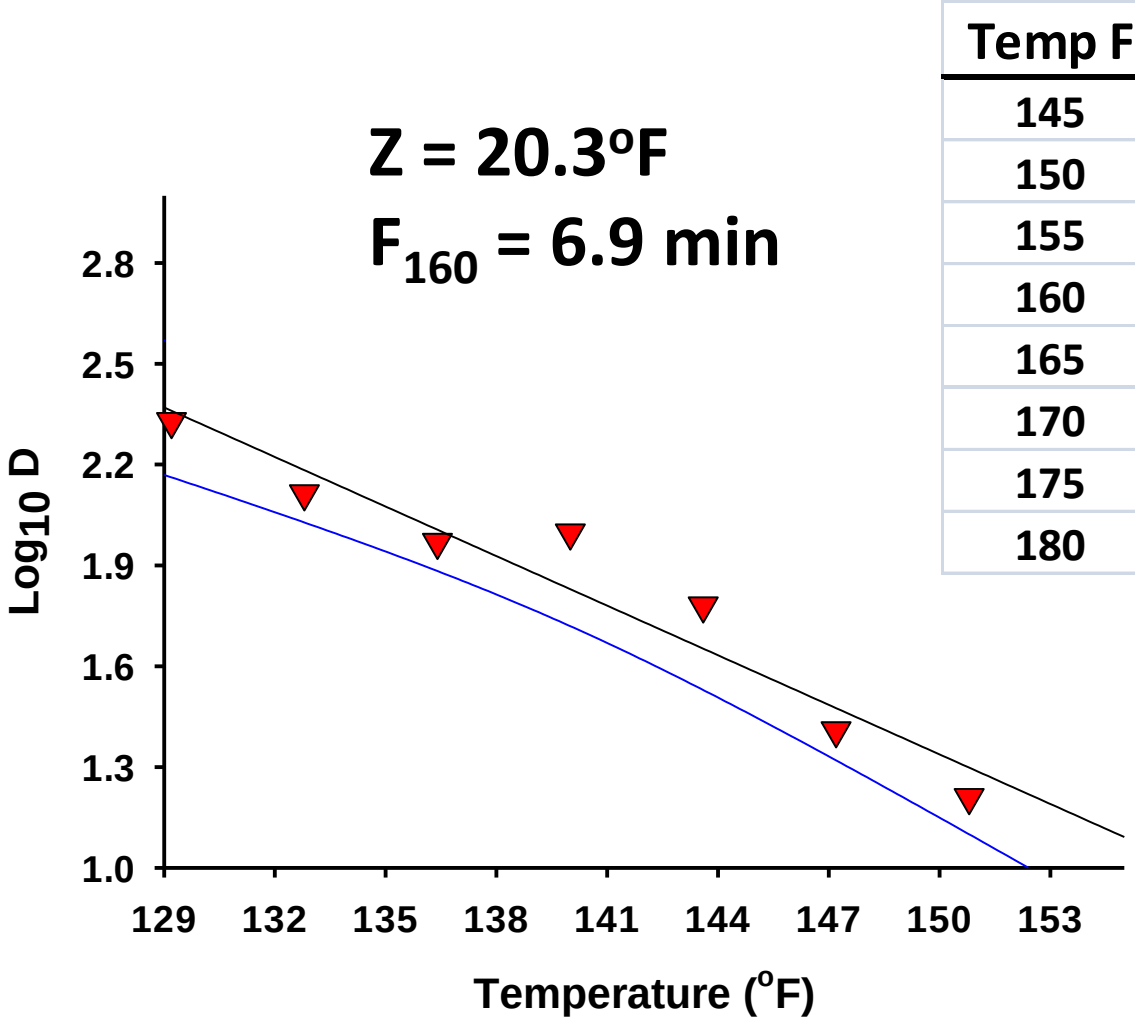
$$\log S = N_0 - 5(\tau/t^*)^{\beta}$$



No	8.30	CFU/ml
B	0.872	
5D value (T*)	24.07	min
Log 5D	1.38	

pH 4.6 Data: Z value determination*

Temp F	pH 4.6	pH4.1
145	37.9	7.1
150	21.5	3.9
155	12.2	2.2
160	6.9	1.2
165	3.9	0.7
170	2.2	0.4
175	1.3	0.2
180	0.7	0.1



pH 4.1 data from Breidt et al., 2010. *Food Prot Trends* 30(5):268-272

*** With 5*SE added**

Next steps…

- pH 4.6 data: times are 5X greater than pH 4.1 data (F_{160} from 1.2 to 6.9 min)
- Data is non-linear.
- TDT data: higher temperatures (160^{o} F or greater) needed for these experiments
- Alternative acids?
- pH alone

Nice, but somewhat skewed

On the drawing board

- Alternative acids, pH 4.6
  - Gluconic acid: *acid independent* data
  - Citric, Phosphoric?
- *Listeria* and *Salmonella*
  - pH 4.1 data showed *Listeria* = EHEC and *Salmonella* was significantly more heat sensitive
- Spore cocktail
  - *Bacillus spp.* (*licheniformis, coagulans*)
  - *Alicyclobacillus*

2. *Bacillus* spores

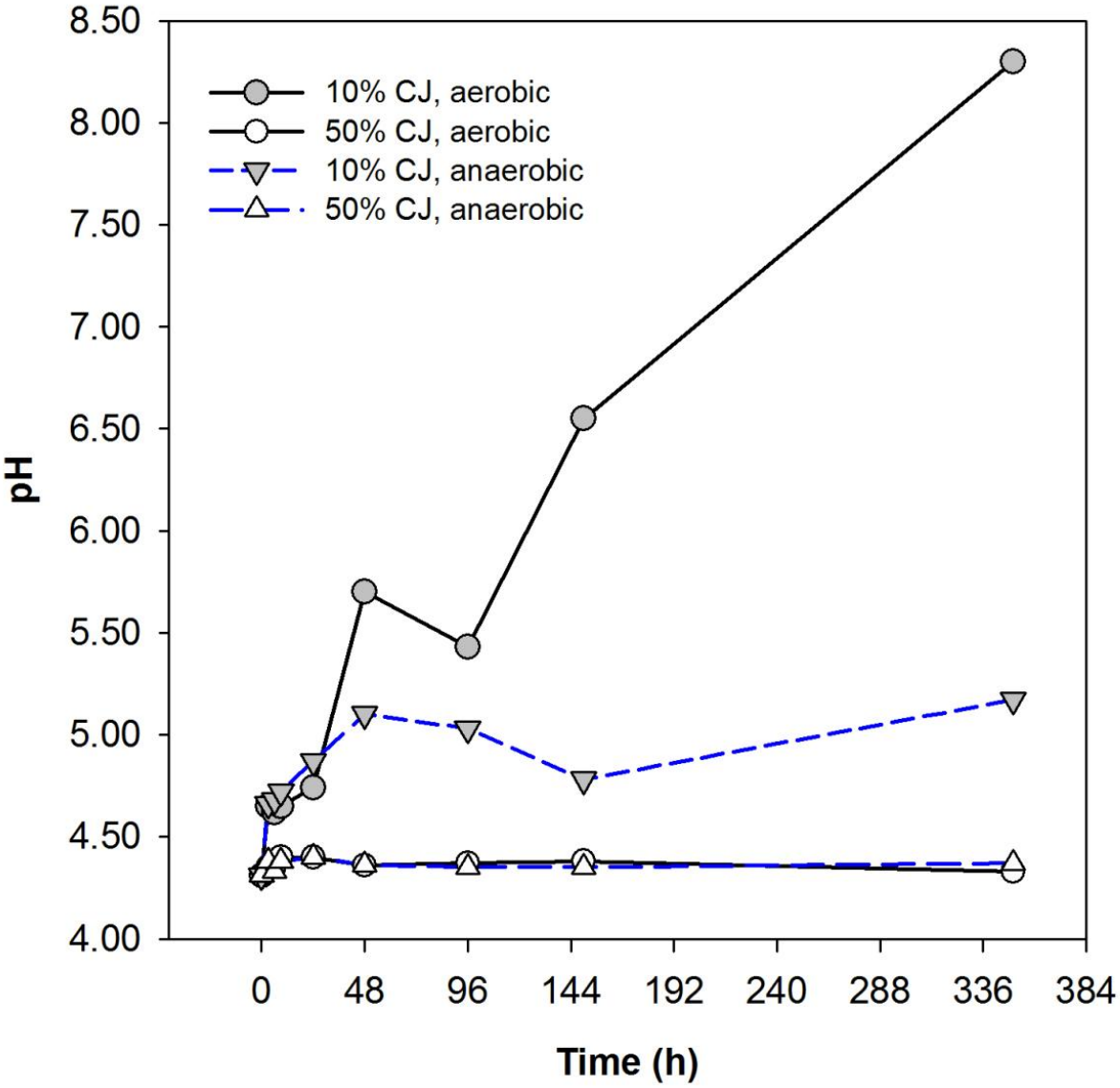
- Targeting acidified products with pH values between 4.1 and 4.6
- What are the limiting pH values for spore germination and growth?
- What is the mechanism of pH rise?
- How much buffer capacity is there to resist pH change in ‘typical’ product formulations?
- How much oxygen required for spore germination?
- TDT data for bacillus spores with conditions typical of acidified foods (not tomato products) at pH 4.6.

pH increase: microbiology and biochemistry

- Microorganisms of interest
  - *Alicyclobacillus* species
  - *Bacillus licheniformis*
  - *Bacillus coagulans*
- Non-inhibitory medium for studies
  - CJ broth
- Survey of pH elevation
  - *B. licheniformis*
- Mechanism: amino acid deamination?
  - HPLC, amino acid analyzer
- Titrations with standardized base to determine buffer capacity

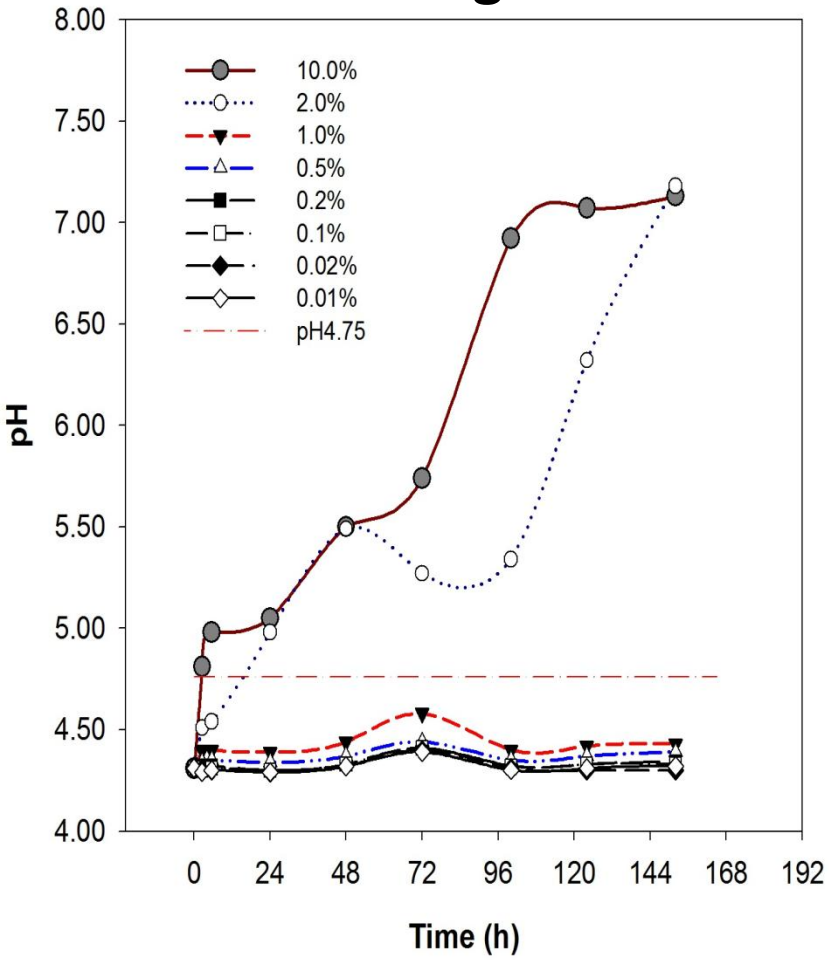
B. licheniformis: Sugar and pH elevation

- Fermentation *vs.* deamination
- CJ has 2% fermentable sugar
- Amino acids are present!

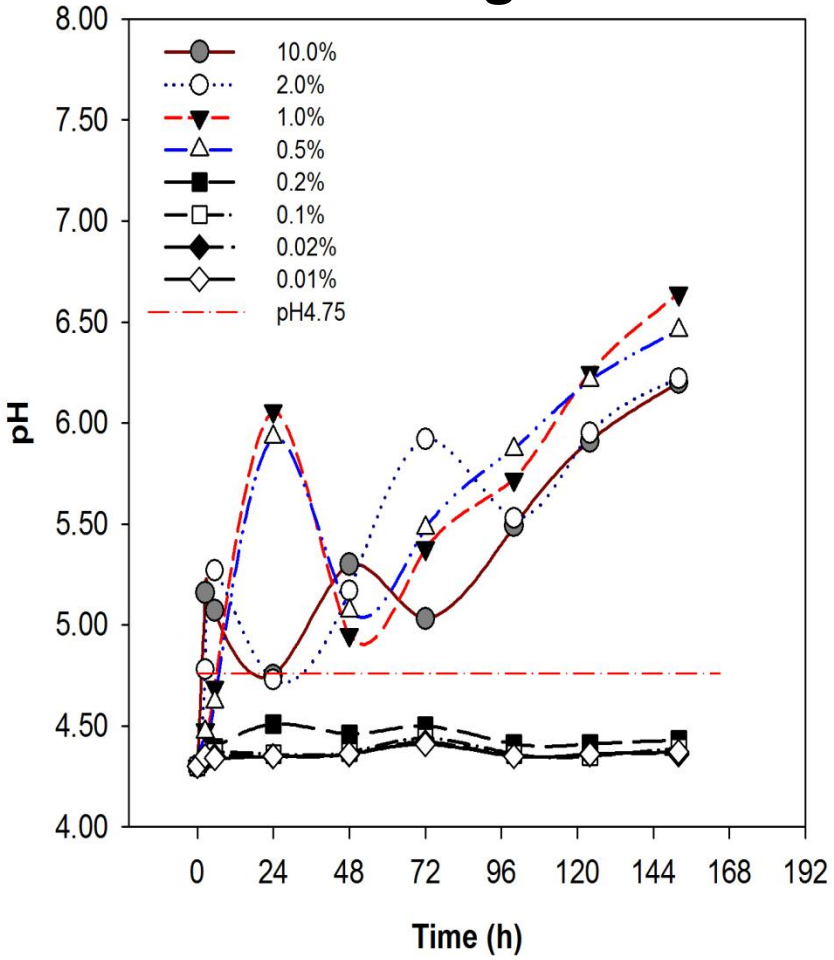


pH Elevation and Arginine

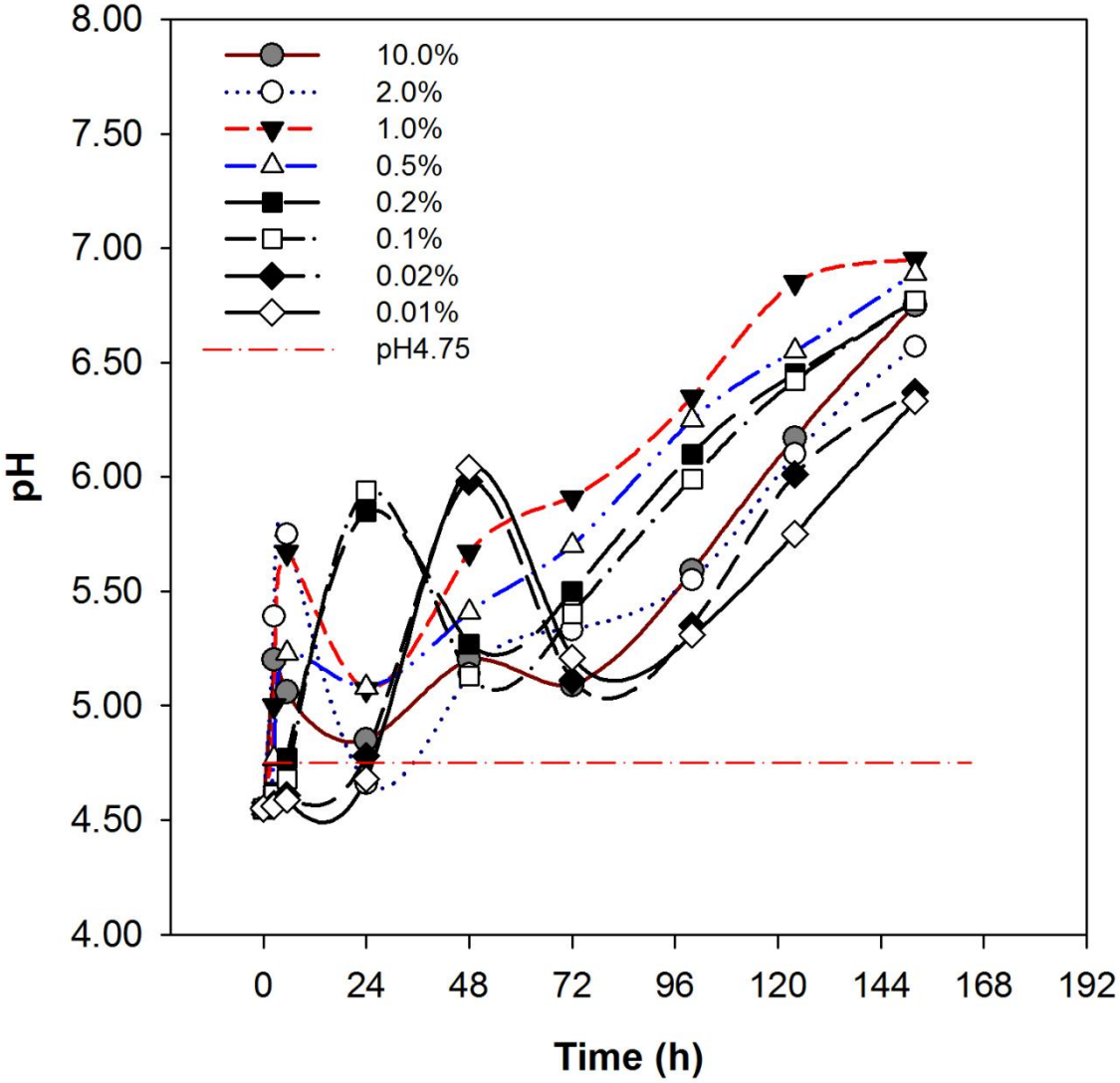
- added arginine



+ added arginine

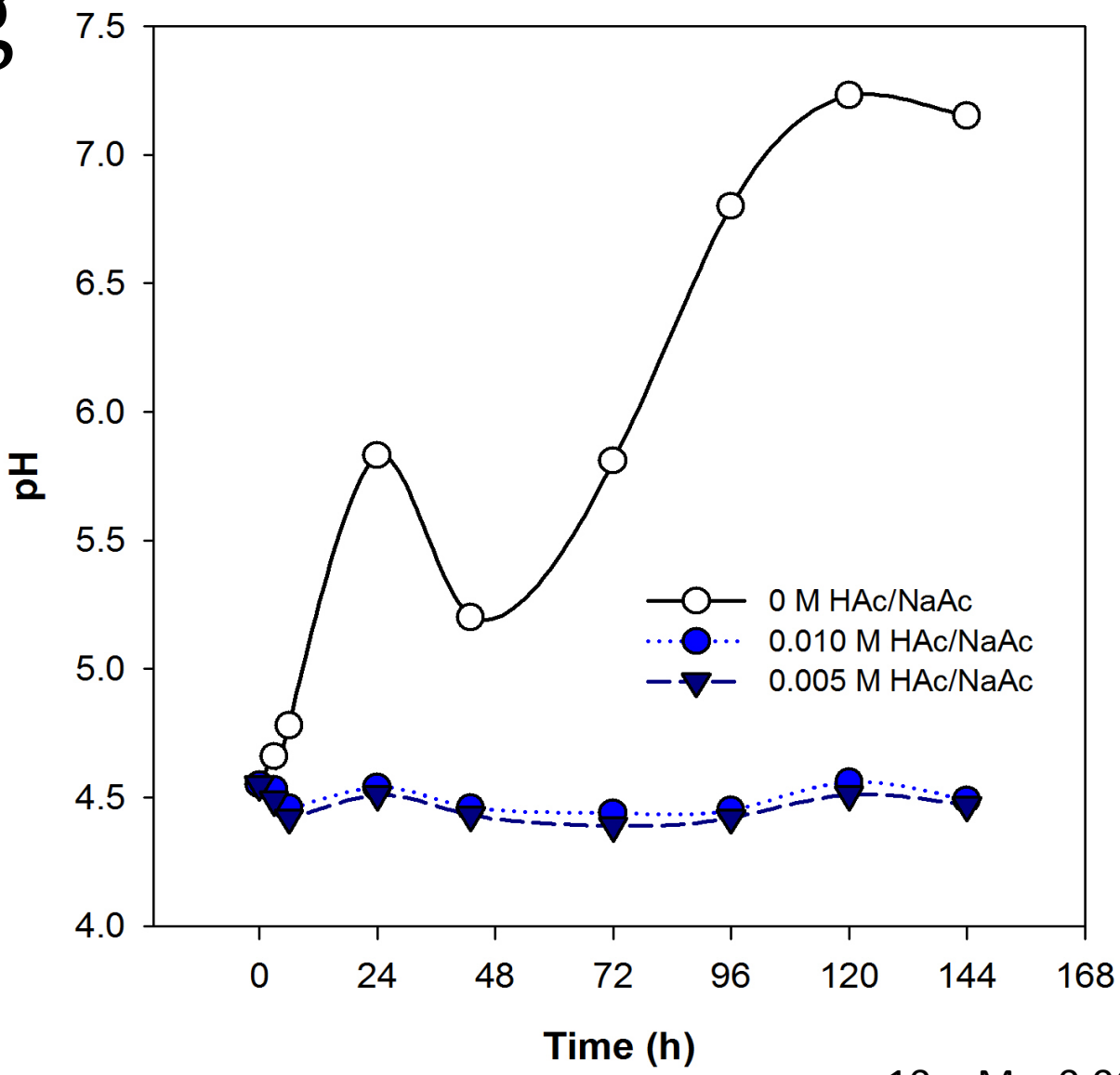


pH 5.5 with Arginine



Buffering

- 10% CJ
- pH 4.55



10 mM = 0.06%

Bacillus results

- Deamination of arginine can result in an initial pH rise
  - Aerobically AND anaerobically, but only if spores germinate and grow (we used vegetative cells)
- Arginine is not the predominant amino acid in CJ but sufficient amount is present
- Other amino acids can be deaminated as well!
- Buffering is important!

Buffer Capacity and pH

- Buffer capacity is the ability of a solution to resist a pH change.
- Cucumber juice has buffering due to acids, bases, and amphoteric compounds.
- Concentration and pKa values
  - Additive
  - Undefined
- Can be determined by titration?
  - Hypothesis: The complex buffering of CJ (and other vegetable based broths) can be modeled as a simple buffer with a single concentration and pK

Buffer Capacity

$$\beta = \frac{\partial C_b}{\partial \mathrm{pH}} = \frac{\partial C_b}{\partial [\mathrm{H}^+]} \frac{d[\mathrm{H}^+]}{d\mathrm{pH}} = -2.303[\mathrm{H}^+]\frac{dC_b}{d[\mathrm{H}^+]}$$

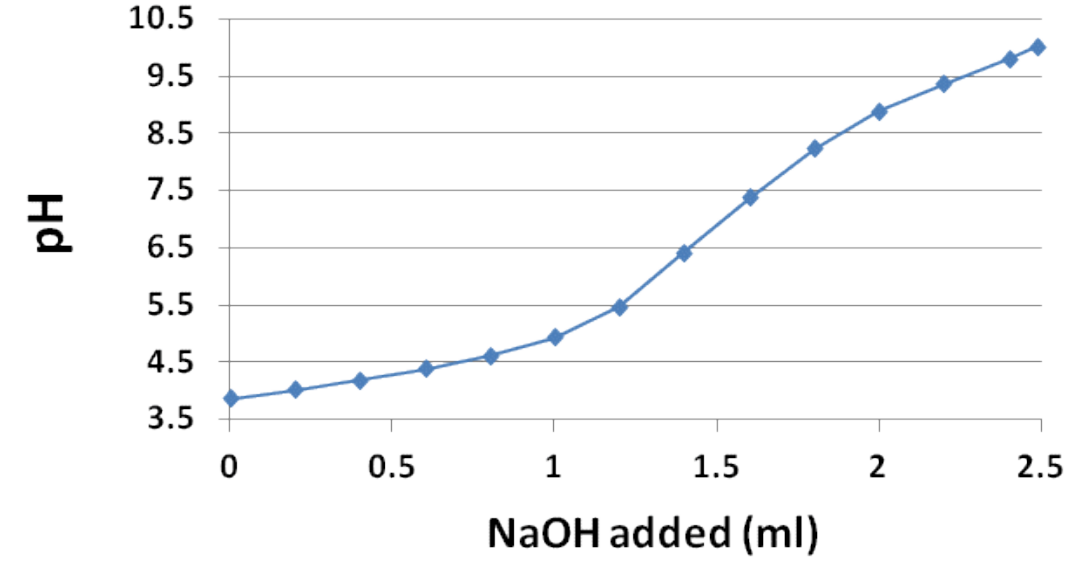
$$\beta = 2.303\left(\frac{CK_a[\mathrm{H}^+]}{([\mathrm{H}^+] + K_a)^2} + \frac{K_w}{[\mathrm{H}^+]} + [\mathrm{H}^+]\right)$$

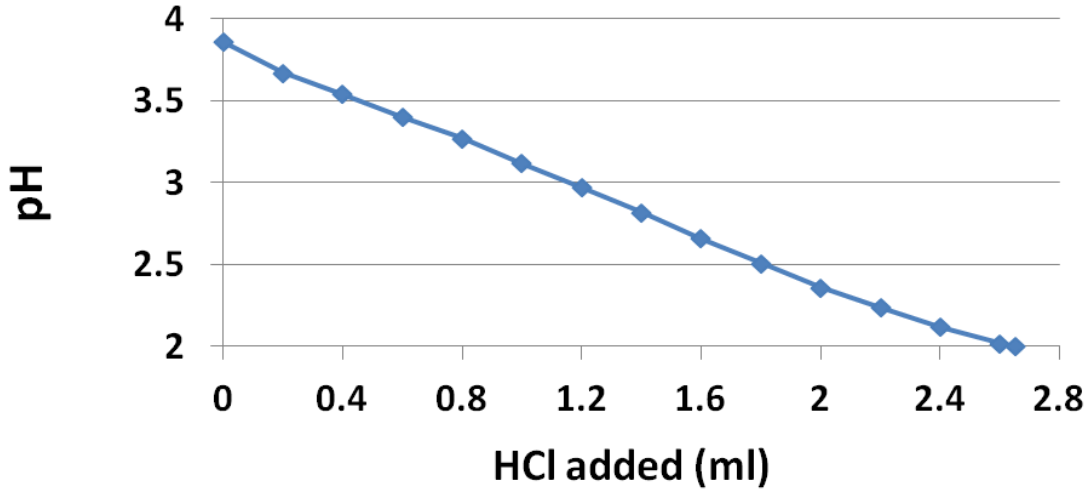
References

Dougherty DP, Ramos Da Conceicao Neta E, McFeeters RF, Lubkin SR, Breidt F Jr. 2006. Semi-mechanistic partial buffer approach to modeling pH, the buffer properties, and the distribution of ionic species in complex solutions. J Agric Food Chem 54:6021-6029.

Butler, J. N.; Cogley, D. R. Ionic Equilibrium: Solubility and pH Calculations; John Wiley and Sons: New York, 1998.

Titration of CJ with acetic acid





Acetic Acid

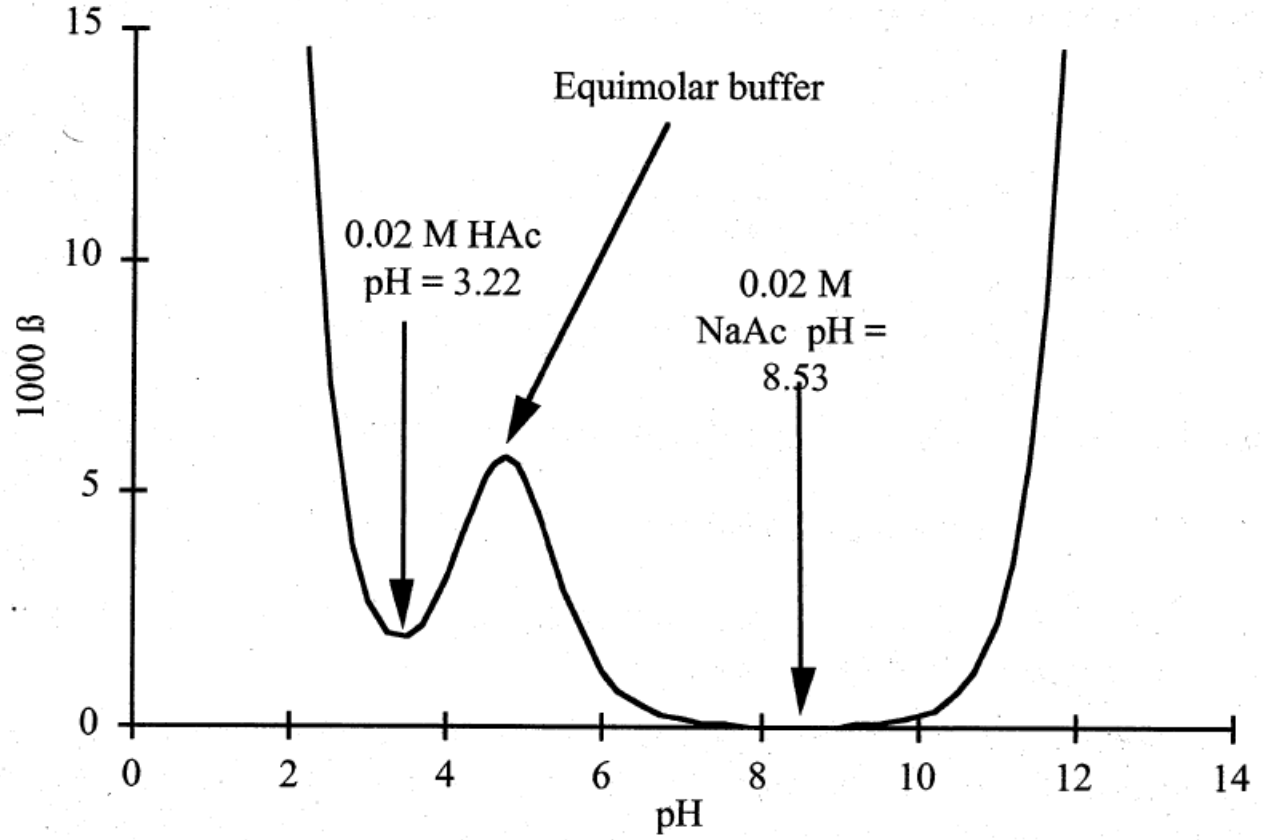
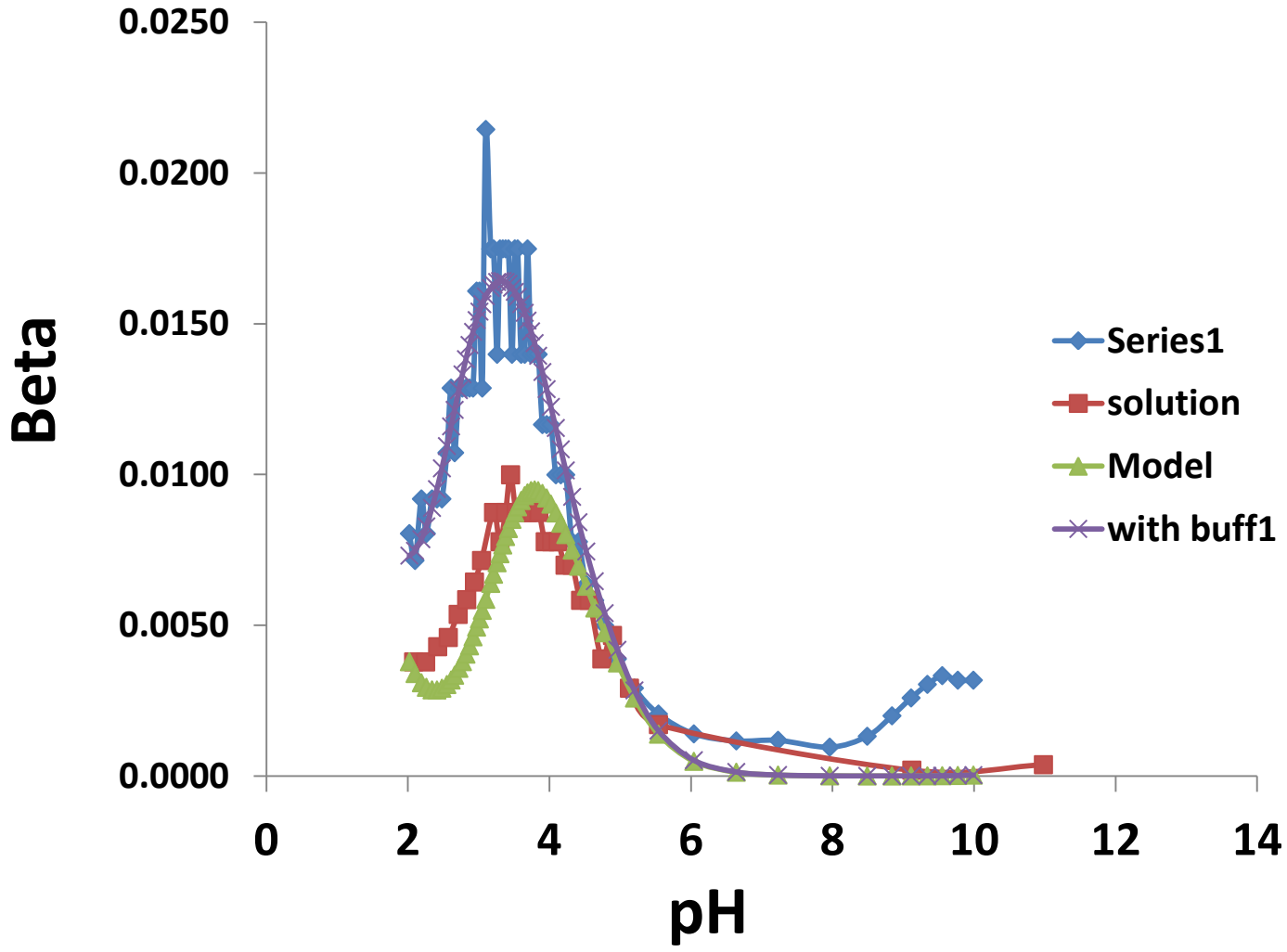


FIGURE 4.22. Buffer index of equimolar acetic acid–sodium acetate as a function of pH.

Butler, J. N.; Cogley, D. R. Ionic Equilibrium: Solubility and pH Calculations; John Wiley and Sons: New York, 1998. P. 135

Buffer capacity data and model



Buffer capacity with hypothetical buffer (pK apx. 3.0)

		M/L	mM	mM	
	pK	Conc	lactic	acetic	NaCl %
FF	2.997	0.125	103.3767	29.57	7.5
7day	2.909	0.066	55.72	27.69	5.2
New	2.843	0.036	11.53	0	6.88

Concentration is proportional to lactic acid concentration (Rsq = 0.98)

- Fermentation brines can be modeled using a buffer with a single pKa = 3.0
- NEXT: Allows predictions of pH change with bacillus growth?

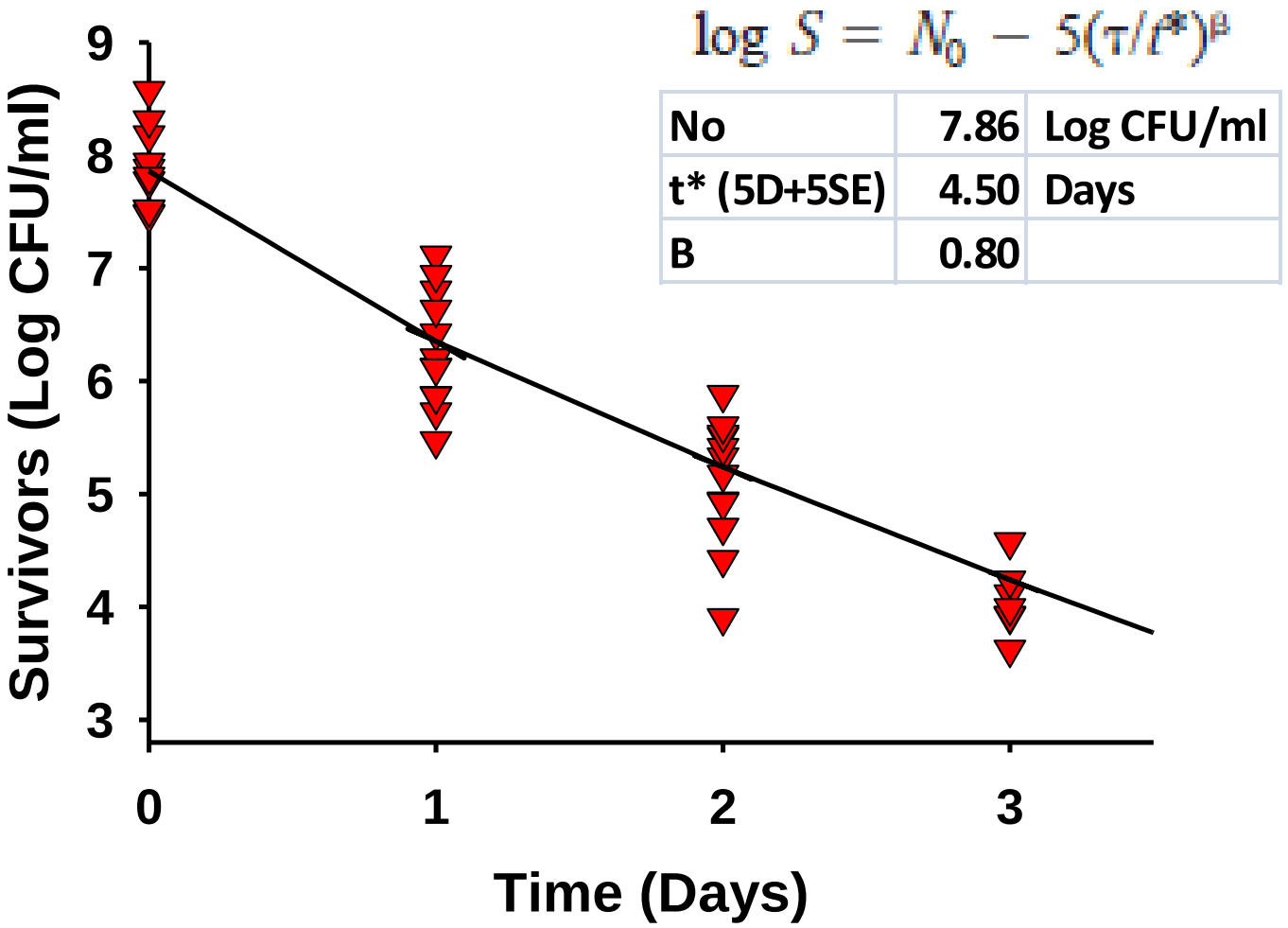
3. Cold fill hold at pH 3.5

- Current data shows requires pH 3.3 or below
  Breidt F, Hayes JS, McFeeters RF. 2007. J Food Prot 70(11):2638-2641
  - CJ as non-inhibitory medium
  - Acetic acid was used as the primary acidulent to get pH at or below pH 3.3
  - At 25^{o}C (77^{o}F): 48 hr.
  - At 10 C (50 F): 6 days
- Alternatives for acidified foods, pH 3.5
  - 2% and 2.5% acetic acid
  - Citric + acetic (1% each?)
  - Phosphoric
  - Preservative acids (benzoate, sorbate, etc.)
  - Fumaric acid

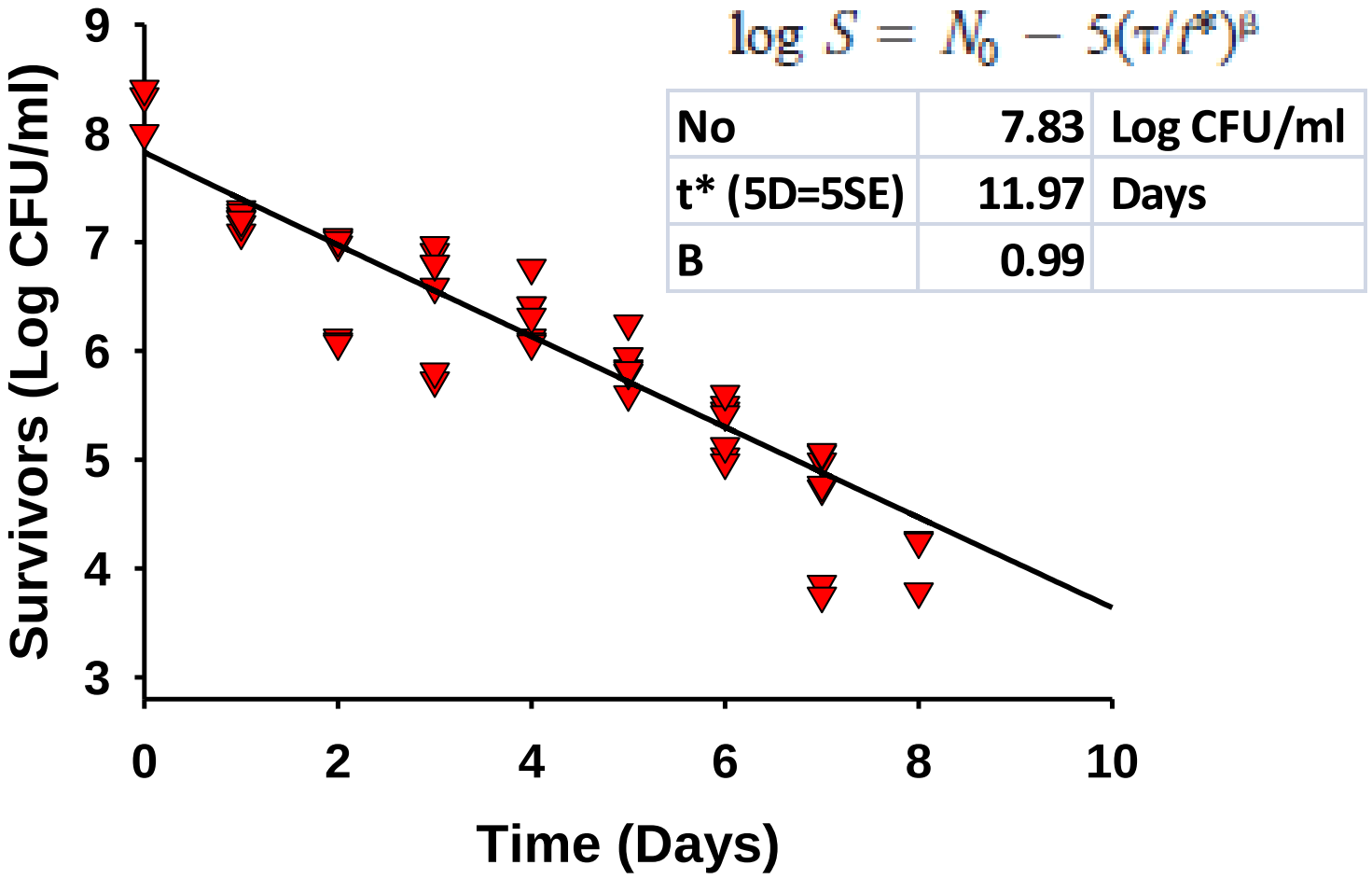
Microbiology

- Cocktail of 5 EHEC strains
  - Growth conditions to induce acid resistance
- Experiments done at 10^{o}C
  - Above refrigeration, low enough to prevent heating
- Inoculate brined cucumbers with indicated acid conditions
  - Sampling through septa using a syringe
  - Oxygen limited (essentially anaerobic)
  - Brined cucumbers are a non-inhibitory vegetable products that can be representative of a variety of products
- Plate on non-selective media
  - Recovery of injured cells
- Independent replication
- Weibull model for 5-log reduction and statistics

Cold-fill-hold pH 3.5, 2.5% Acetic



Cold-Fill-Hold pH 3.5, 2% Acetic



Conclusions

- Thermal processing at pH 4.6
  - With 100 mM acetic acid pH 4.6: F_{160} = 6.9 min., Z = 20.3^{o}F.
- Cold fill hold pH 3.5
  - Data for 2.5% acetic acid, apx. 5 days for holding!
  - 2%: apx. 12 days

  NOTE: pH 3.3 data, 6 days at 50^{o}F or 48 hr. at 77^{o}F
- Spore forming bacilli
  - IFT abstract: “pH elevation by *Bacillus licheniformis* in Acidified Vegetable Broth” by Meng et al.
  - pH 4.2 was lower limit for increase
  - Glucose represses pH rise, oxygen required!
  - Deamination of arginine responsible for early pH rise

More conclusions

- *Salmonella* and *Listeria* have previously been shown to be less acid resistant
  - Selected trials will be done to confirm this…
- pH 3.5 with 2.5% acetic acid
  - Holding 5 days at 10^{o}C or above
  - 2% acetic acid is probably not useful
- Additional acids and conditions will be done…
  - Suggestions? Fred.Breidt@ars.usda.gov
  - Objective is to meet a wide variety of products with least number of experiments
- White paper/publications with new data being prepared!

United States Department Of Agriculture
Agricultural Research Service

ARS Food Science Research Unit
NC State University, Raleigh, NC
http://ncsu.edu/foodscience/USDAARS/

Fred.Breidt@ars.usda.gov

THE MICROBIAL WORLD…

Staff, Students and Collaborators

Ms. Jane Caldwell
Ms. Katherine Kay
Ms. Kelly Fletcher
Ms. Catherine Xia Meng
Dr. Deog Hwan Oh
Dr. Zhenquan Yang
Dr. David Muddiman
Dr. Suzanne Johanningsmeier
Dr. Fletcher Arritt
Dr. Hosni Hassan
Dr. Barbara Ingham
Dr. David Green
Dr. Glenn Black